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Two new African *Pulveroboletus* with ornamented spores

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Abstract — Two new bolete species, *Pulveroboletus bembae* and *P. luteocarneus*, are described from the rain forest of Gabon. Both are characterized by basidiospores with a similar rough structure.

Key words — *Boletales*, taxonomy

Introduction

Pulveroboletus was created by Murrill (1909) and is typified by *Boletus ravenelii* Berk. & M.A. Curtis. One hundred years later the circumscription of the genus remains controversial. *Pulveroboletus* is an essentially tropical or subtropical genus with velangiocarpic basidiomes producing an olivaceous brown spore deposit. The velar layers are pulverulent and detersile, sometimes viscid when moistened, and consist of narrow and encrusted hyphae (Pegler & Young 1981).

Singer (1962) greatly extended the concept of *Pulveroboletus* in a way Corner (1972) found confusing. In fact, most of the species added by Singer to the genus have no veil at all, not even a marginal one, and are characterized by a gelatinization of the epicutis. According to Corner, the continuity between the type species (*P. ravenelii* (Berk. & M.A. Curtis) Murrill) with a fibrillose, pulverulent-arachnoid veil, and the other sections is unclear, and the diagnostic characters are not sufficient to separate *Pulveroboletus* from subgen. *Boletus* (Corner 1972: 10). Singer replied to Corner's contention by underlining that *P. umbilicatus* (Massee) Singer—with a dry piloso-fasciculate tomentum when young turning mucilaginous with age—could be considered as the transition from the dry to the glutinous species (Singer 1986: 772). He then confirmed the seven sections he previously defined within the genus.

Until now, 12 species of *Pulveroboletus* are described in tropical Africa, mostly from DR Congo (Heinemann 1951, 1964). Six additional taxa collected

in the miombo woodland of DR Congo (Heinemann 1964) and Zambia (Watling & Turnbull 1992) remain unnamed.

Materials and methods

The macroscopic descriptions are based on field notes and photographs taken by the first author. Codes (between square brackets) and names for colours correspond to the Methuen Handbook of Colour (Kornerup & Wanscher 1983).

The microscopic structures were observed in Melzer's reagent as well as in congo-red ammonia. Measurements were performed using an Olympus BX51 light microscope, with digital camera and AnalySIS® Five imaging software (Soft Imaging System GmbH). Mean values (in italics) ± 1.96 × standard deviations, and minimum-maximum values (between brackets) are given for all microstructures and derived parameters (length/width ratios). For the statistical data the number N of basidiospores, basidia, and cystidia that were measured is given (between braces). For the analysis with the scanning electron microscope (SEM), small parts of the tubes were taken from the exsiccata. They were pretreated for 12 hours in water and ammonia. After a period of 1 hour in 70% ethanol, they were passed twice for 30 minutes in dimethoxymethane, before being submitted to the process of critical point drying. The samples were sputter-coated with gold (using Argon-gas, under 0.05 mbar) for 3.5 hours, until a layer of approximately 15 nm was obtained. The scanning electron microscopy was then carried out with a JEOL 5800 LV with a tension of 25 kV and working distance of 10–12 mm. Digital SEM-images were captured using Orion V (vers. 5.22) Image Management System. The holotype material and the additional collections are deposited at the National Botanic Garden of Belgium's herbarium 'BR' (abbreviation following Holmgren et al. 1990).

Taxonomy

Pulveroboletus bembae Degreef & De Kesel, **sp. nov.** FIGURES 1–3

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Pileus 30–35 mm, convexus dein convexo-planus, interdum subumbonatus, concolor badius ad brunneo-ferrugines, siccus, levis vel impolitus dein aliquantum conluceus; margine praeter pallidus, cum velamine, levi dein rimuloso. Tubi 4–5 mm, adnati, depressi ad stipitem, subventricosi, pallido-flavi, immutabiles. Pori angulares vel rotundi, denique 1 mm, concolores, griseo-flavi vel subpallidi, immutabiles. Stipes 37–55 × 4–5 mm, cylindricus, ad basim angustior 2–4 mm, solidus dein subfistulosus, impolitus, siccus, pallido-brunneus, vix subfurfuraceus, cum minusculis squamulis badiis tectus; mycelium basale flavum. Caro crassa, sufflava, virgata badia, pallido-brunnea in basim stipitis, immutabilis. Annulus levis, tenuis, praeter ad stipitem vel marginem pilei, ephemerus, albus. Basidiosporae (9.3–11.3 × 3.9–4.7 µm) subfusiformes vel boletiformes, scruposae, inamyloideae. Basidia (26.9–39.3 × 9.0–12.0 µm), cylindrica vel subclavata, tetraspora.

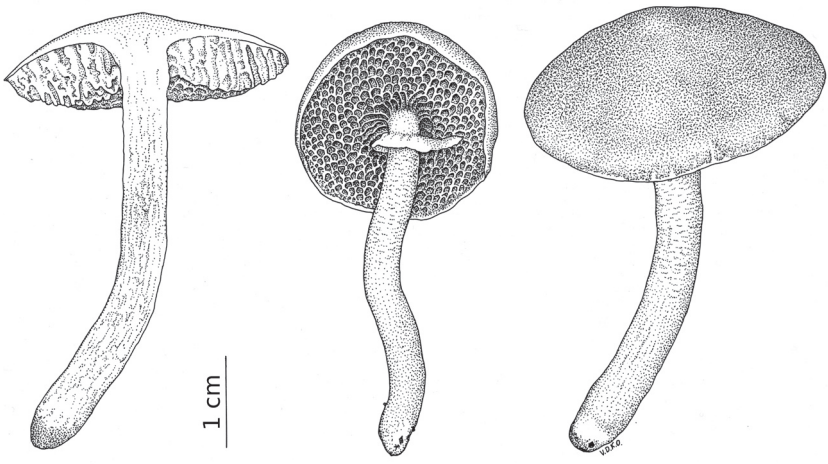

Fig. 1. *Pulveroboletus bembae* (Degreef 643, holotype). Basidiomes.

Pleurocystidia (57.4–92.6 × 9.4–17.4 µm), fusiformia, hyalina. Cheilocystidia (50.6–75.1 × 12.2–16.1 µm) fusiformia, crebra, hyalina. Trama phylloporoidea, sine mediostrata. Pileipellis physalo-palisadodermiformis; elementa terminalia brunnea, inflata vel spheropedunculata (25–45 × 20–40 µm); subpellis ex hyphis brevibus anticlinalem formantibus (20–40 × 5–8 µm). Elementa terminalia squamularum stipitis pileique 15–30 × 10–15 µm. Fibulae desunt.

Holotypus: Africa, Gabon, Woleu-Ntem Province, Bitouga, 8.IV.2008, Degreef 643 (BR).

Etymology: from 'bemba', name given by the Baka pygmies to the tree *Gilbertiodendron dewevrei* that is associated with the species.

Pileus 30–35 mm in diameter, convex, sometimes subumbonate, becoming plano-convex; almost uniformly rust-brown to reddish brown [6–8E6], with a slightly paler margin when young; surface dry, dull, becoming slightly shiny with age, rimulose towards the margin in older specimens; margin with a slightly exceeding pileipellis, deflexed, partly beset with velar remnants; context at the centre of the pileus < 4–5 mm thick, becoming very thin towards the margin, cream-colored to pale yellow [4A3] with pale reddish brown to light brown [7D4–5] shades under the pileipellis and down the stipe. Tubes subventricose, moderately depressed around the stipe, adnate, rarely with a decurrent tooth, < 5 mm long, greyish yellow [4B3–4C3] to mustard yellow [3B6]. Pores angular to round, more elongated around the stipe, < 1(–2) mm in diameter; concolorous with the tubes or a shade greener. Stipe 37–55 × 4–5 mm, central, cylindrical, with a narrow base 2–4 mm, sometimes with yellow [4A3] basal mycelium; solid, becoming stuffed to subfistulose with age; surface dull, dry,

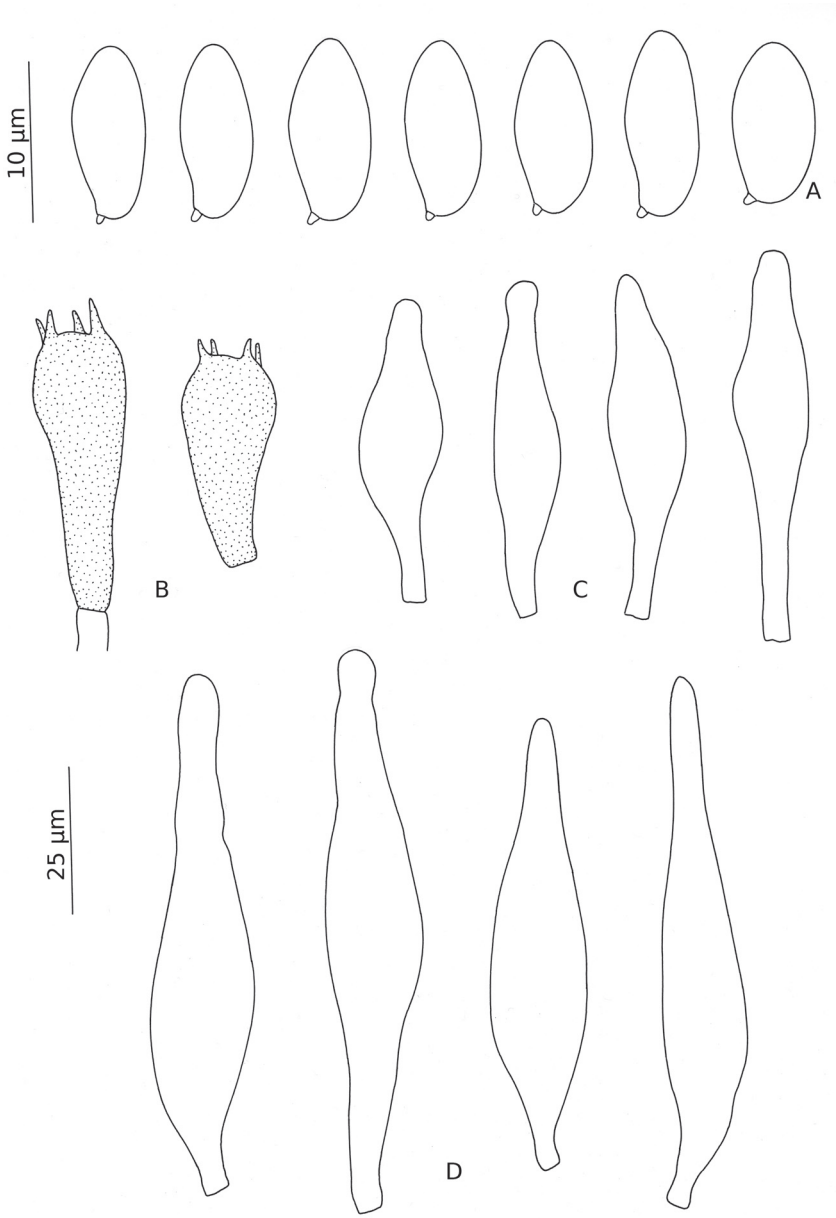

Fig. 2. *Pulveroboletus bembae* (Degreef 619). A. basidiospores; B. basidia; C. cheilocystidia; D. pleurocystidia. (A: scale bar = 10 µm; B–D: scale bar = 25 µm).

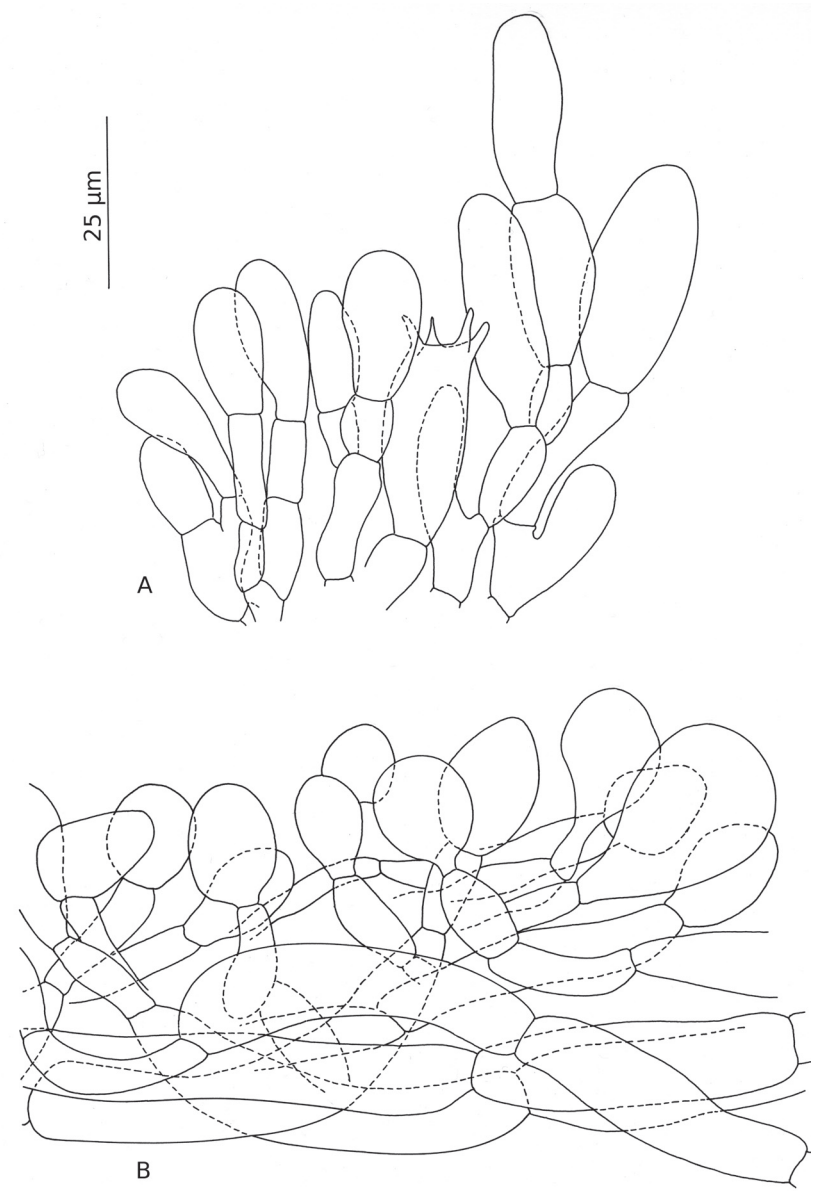

Fig. 3. *Pulveroboletus bembae* (Degreef 619). A. stipe squamule; B. pileipellis (profile views).

pale brown, entirely covered with minute brown to reddish brown [6–7D6–7] squamules; context cream-colored, streaked with pale reddish brown to light brown [7D4–5] from the upper third towards the base, the base light brown [7D4–5]. Annulus either on the stipe or the margin of the cap, very fine, wooly, whitish yellow, fragile and fugacious, usually lost in older specimens. Odour mildly fungoid to earthy. Taste mildly fungoid. Spore print not observed. Mycelium yellow.

Basidiospores (9.1–)9.3–*10.3*–11.3(–12.1) × (3.5–)3.9–*4.3*–4.7(–5.3) µm, l/w = (1.88–)2.13–*2.42*–2.71(–2.86) {N=48}, subfusiform, boletoid, with a pronounced suprahilar depression, weakly pigmented and rough (under SEM), inamyloid. Basidia (28.5–)26.9–*33.1*–39.3(–39.1) × (8.9–)9–*10.5*–12(–11.8) µm {N=24}, cylindrical to narrowly clavate, hyaline, with 4 sterigmata. Pleurocystidia (64.6–)57.4–*75*–92.6(–95) × (9.9–)9.4–*13.4*–17.4(–17.4) µm {N=21}, fusiform, moderately frequent, emergent, thin-walled, concolorous with the hymenium, hyaline, without crystals or encrustations. Cheilocystidia (52.2–)50.6–*62.9*–75.1(–73,9) × (11.7–)12.2–*14.1*–16.1(–15.6) µm {N=21}, fusiform, abundant to crowded, thin-walled, hyaline, without crystals or encrustations. Pileipellis a thin physalo-palisadoderm with short anticlinal hyphae of 20–40 × 5–8 µm supporting one or two inflated, brownish, globose to spheropedunculate terminal elements of 25–45 µm across, non-amyloid, thin-walled, without encrustations; mucilaginous layer not seen. Stipitipellis smooth parallel hyphae; squamules with a physalo-palisadodermic construction with short anticlinal elements supporting elongated inflated elements of 15–30 × 10–15 µm and some scattered basidia. Trama composed of hyaline, thin-walled hyphae, 10–15 µm wide, parallel, without a mediostratum. Clamp connections absent.

Ecology and distribution: guineo-congolian rainforest dominated by *Gilbertiodendron dewevrei* (*Caesalpinioideae*), growing in small groups.

Specimens examined– GABON. Ogooué-Ivindo Province, Ipassa-Makokou Research Station, N0°30'15" E12°46'49", 2.IV.2008, *Degreef* 601. Woleu-Ntem Province, Minkébé National Park, near Minvoul, N02°09'18" E12°08'07", 6.IV.2008, Degreef 619. Ibid., Bitouga, N02°07'08" E 12°07'42", 8.IV.2008, Degreef 643 (**Holotype**-BR).

Pulveroboletus luteocarneus Degreef & De Kesel, **sp. nov.** Figures 4–6

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Pileus 20–35 mm, convexus dein convexo-planus, rubro-brunneus, siccus, levis et tomentosus dein floccoso-squamosus et rugulosus, semper pallide ruber in fractu, margine praeter pallide ruber. Tubi 1 cm longi, adnati, subventricosi, griseo-lutei, immutabiles. Pori angulares, 0.5–0.7mm, rubro-brunnei, immutabiles, pulverulenti. Stipes 35–80 × 2–3 mm, procerus, cylindricus, impolitus, siccus, rubro-brunneus, subfurfuraceus, cum flocca rubro-brunnea tectus. Caro crassa, griseo-lutea, statim aurantiacescens. Basidiosporae

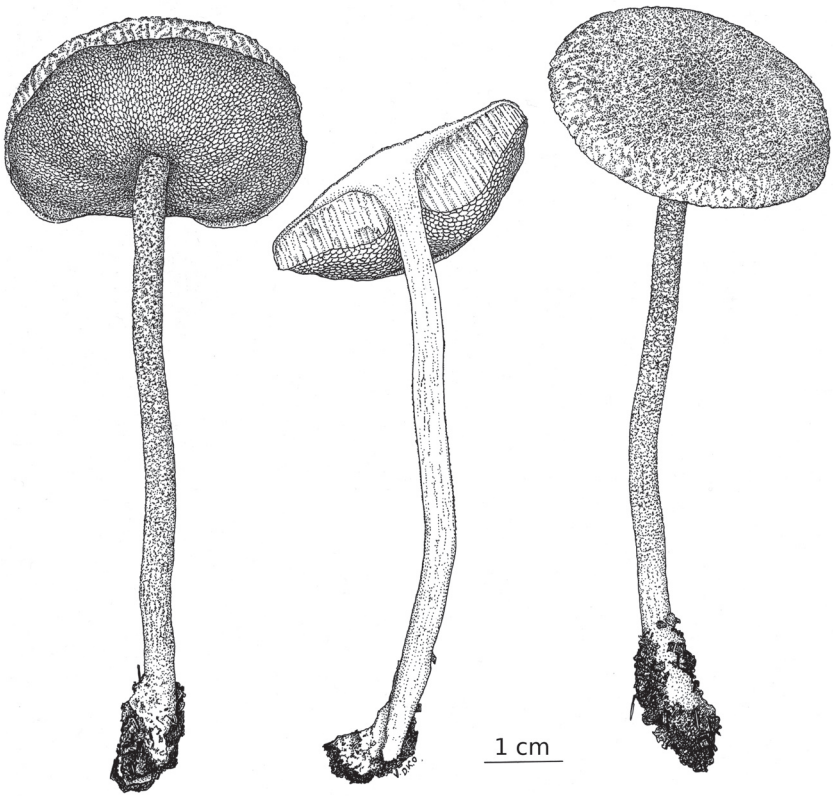

Fig. 4. *Pulveroboletus luteocarneus* (Degreef 632, holotype). Basidiomes.

(11.2–14.7 × 4.5–5.7 µm) subfusiformes vel boletiformes, scruposae, inamyloideae. Basidia (21.8–31.4 × 9.3–12.3 µm), subclavata, tetraspora. Pleurocystidia (36.7–73.4 × 8.5–14.4 µm), fusiformia, crebra, lutea, inamyloidea. Cheilocystidia (38.3–63.2 × 6.9–11.8 µm), fusiformia, lutea, inamyloidea. Trama tubi subboletoidea dein phylloporoidea, parallela, sine mediostrata. Pileipellis trichodermiformis; elementa terminalia rubro-brunnea, incrustata, cylindracea (30–80 × 15–20 µm). Squamulae stipitis rubro-brunneae, hymeniformes; elementa terminalia rubro-brunnea, levia, angusto-clavata, 50–85 × 12–20 µm. Fibulae desunt.

Holotypus: Africa, Gabon, Woleu-Ntem Province, Minkébé National Park, Doumassi, 7.IV.2008, Degreef 632 (BR).

Etymology: refers to the context turning yellow orange after bruising.

Pileus 20–35 mm in diameter, convex, becoming plano-convex; surface dry, dull, tomentose at first, coarsely tomentose and rugulose with age, moderately floccose-scaly near the margin, entirely brownish red at first [8F8, 8–9C5], later

pinkish or reddish white [7A2] in the ruptures and scars of the suprapellis; margin even to rugose, with slightly exceeding pileipellis, deflexed, without velar remnants except for a reddish pulverulence along the margin (magnifying glass); context at the centre of the pileus < 3–4 mm thick, fleshy, gradually becoming thin towards the margin, greyish red [9B4] at first, later reddish orange [7A6] with darker shades under the pileipellis and above the tubes. Tubes ventricose, adnate, without decurrent tooth, < 1 cm long, brownish orange [7C3] when young, soon becoming yellowish grey [4B2]. Pores mostly angular, regular, less than 0.5–0.7 mm in diameter, not staining when bruised, reddish brown to almost concolorous with the cap [9D5–9F8], with reddish-orange [7A–B7] encrusted velar remnants, persistent in older specimens as a red powdery pulverulence (sometimes scanty along margin of the cap). Stipe 35–80 × 2–3 mm, central, slender, cylindrical, terete, with normal to subbulbous base, without annulus, at first near the apex with reddish-orange [7A–B7] encrusted velar remnants, later locally pulverulent; surface dull, dry, entirely floccose-scaly, upper part brownish red [9C6] to reddish brown [9E5], pale greyish yellow [4A2–4B2] towards the base and in between the ruptures; context fibrous, stuffed, becoming hollow with age, beige [4A2], immediately becoming orange [5A5] when exposed. Odour and Taste weak, not distinctive. Spore print greyish yellow [4B4–4C4]. Mycelium yellow.

Basidiospores (11.3–)11.2–*13*–14.7(–15.6) × (4.4–)4.5–*5.1*–5.7(–5.7) µm, l/w = (2.09–)2.24–*2.54*–2.84(–2.95) {N=65}, subfusoid, boletoid, with a distinct suprahilar depression in profile, weakly pigmented and rough (under SEM), inamyloid. Basidia (23.2–)21.8–*26.6*–31.4(–32.5) × (9.3–)9.3–*10.8*–12.3(–12) µm {N=13}, clavate, hyaline, with 4 sterigmata. Pleurocystidia (38.8–)36.7–*55*–73.4(–81.4) × (7.8–)8.5–*11.5*–14.4(–15.3) µm {N=42}, abundant, narrowly fusiform to fusiform, sometimes clavate, emergent, thin-walled, with yellowish intracellular pigment (persistent in ammonia), without crystals or encrustations, inamyloid. Cheilocystidia (39.2–)38.3–*50.8*–63.2(–62.5) × (6.5–)6.9–*9.3*–11.8 (–11.1) µm {N=24}, similar to pleurocystidia, narrowly fusiform to fusiform. Pileipellis a trichoderm without mucilaginous layer, soon collapsed, forming a thin layer of matted parallel hyphae; terminal elements 30–80 × 15–20 µm, sparsely but distinctly encrusted, non-amyloid and thin-walled. Stipitipellis smooth parallel hyphae (stipe trama) supporting a caulohymenium, soon rupturing into floccose granules composed of densely tufted, elongate to club-shaped elements 50–85 × 12–20 µm, the latter thin walled, smooth, not encrusted, with distinct intracellular pigment; basidia not seen. Veil (partial) composed of non-persistent hyphae, heavily encrusted with reddish-orange crystals; the latter slowly dissolving in water and alkali (persistent in exsiccata, on pores and cap margin). Trama composed of hyaline, thin-walled hyphae, 8–12 µm wide, sub-boletoid at first, soon becoming parallel, with slightly

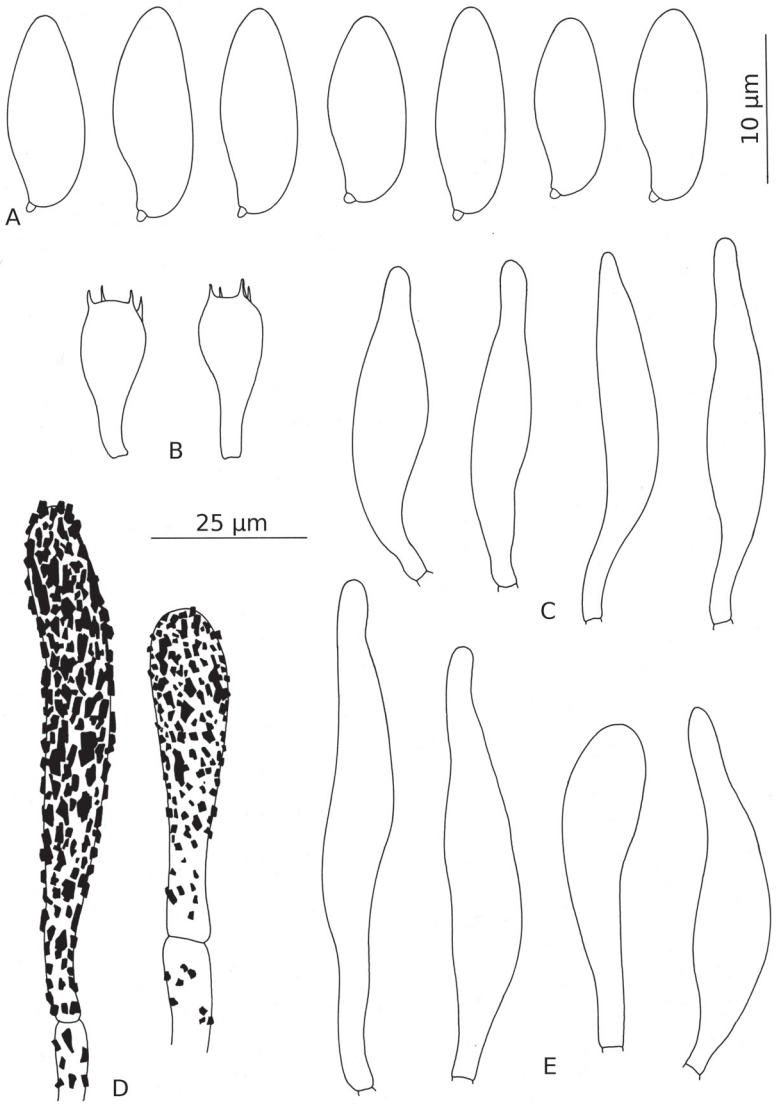

Fig. 5. *Pulveroboletus luteocarneus* (Degreef 632, holotype).
A. basidiospores; B. basidia; C. cheilocystidia;
D. incrusted velar remnants on the pores; E. pleurocystidia.
(A: scale bar = 10 µm; B–E: scale bar = 25 µm).

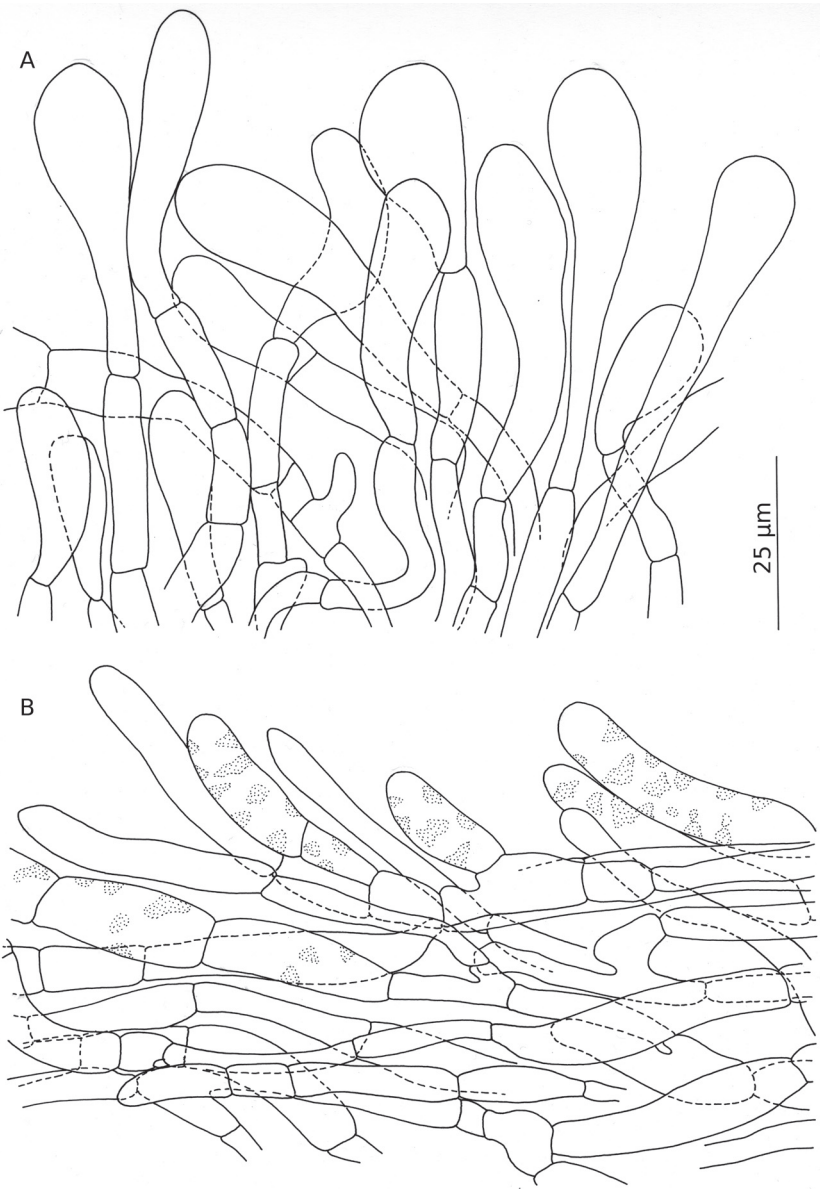

Fig. 6. *Pulveroboletus luteocarneus* (Degreef 632, holotype).
A. fibrils on upper stipe; B. pileipellis (profile views).

narrower hyphae 5–10 µm in the centre, without differentiated mediostratum. Clamp connections absent.

Ecology and distribution: guineo-congolian rain forest, growing solitary under *Uapaca guineensis* (*Euphorbiaceae*) or *Gilbertiodendron dewevrei* (*Caesalpinioideae*).

Specimens examined– GABON. Ogooué-Ivindo Province, Ipassa-Makokou Research Station, N0°30'04" E12°48'24", 24.III.2005, Degreef 315. Ibid., Kongou waterfalls, N0°17'24" E12°34'18", 9.IV.2006, Degreef 395. Woleu-Ntem Province, Minkébé National Park, Doumassi, N2°05'05" E12°10'10", 7.IV.2008, Degreef 632 (**Holotype**-BR) & 634.

Discussion

Whatever the circumscription of *Pulveroboletus* considered, i.e. restricted (sensu Murrill 1909) or enlarged (sensu Singer 1986), obviously both species described are presently best accommodated in the genus. In the classification scheme proposed by Watling (2008), both species key out under *Pulveroboletus*. Despite the overall appearance of the basidiomes that remind *Xerocomus* Quél., the presence of a detersile and powdery veil excludes this possibility. Considering the combined features, i.e. their ectomycorrhizal nature, the olivaceous component in the spore print and the phylloporoid tube trama (initially sub-boletoid), there is little doubt that *Pulveroboletus bembae* and *P. luteocarneus* belong in sect. *Pulveroboletus* sensu Singer. Compared to *P. ravenelii*, both new taxa lack the vivid yellow colour, the bluing context, and the prominently developed veil.

Pulveroboletus luteocarneus resembles *P. carminiporus* Heinem. It is easily separated from *P. carminiporus* by its changing orange context (non bluing), the presence of reddish-orange encrusted velar hyphae (upper part of the stipe and pores), and the ornamented spore surface (smooth in *P. carminiporus*). The taxonomic placement of *P. carminiporus* is still controversial. Singer (1986) placed the species under *Boletus*, in Section 1. *Subpruinosi* Fr. emend. Singer. There is very little against this, as the holotype (*Goossens-Fontana* 718, BR) fits the section's description and shows no clear evidence of a pulverulent veil. It could belong to *Xerocomus*.

Pulveroboletus bembae is morphologically close to *P. annulatus* Heinem. and *P. croceus* Heinem. There is some debate around the identity of the latter two species. Heinemann (1951) suspected their synonymy and Corner (1972), conversely, suggested a difference in carpogenesis (gymnocarpic in *P. croceus*). *Pulveroboletus bembae* clearly differs from both *P. annulatus* and *P. croceus* by its much larger hymenial cystidia (< 95 µm for pleurocystidia compared to maximum 75 µm in *P. annulatus* and *P. croceus*), cream-colored context with pale reddish brown to light brown shades under the pileipellis (evenly white

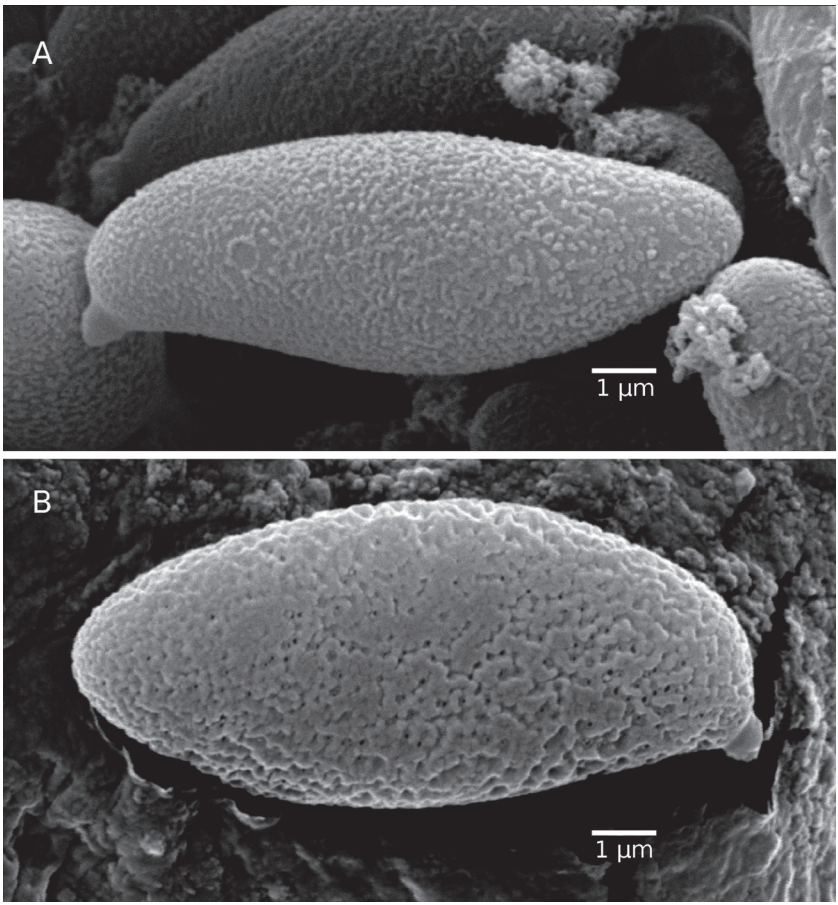

Fig. 7. SEM photographs of basidiospores.
A. *Pulveroboletus bembae* (Degreef 619); B. *Pulveroboletus luteocarneus* (Degreef 632, holotype).

in *P. annulatus* and *P. croceus*) and yellow mycelium (white in *P. annulatus* and *P. croceus*). Moreover, *P. bembae* has a different ecology growing in groups associated with *Gilbertiodendron dewevrei* in the rain forest, whereas *P. annulatus* and *P. croceus* occur solitary in the dry forest. It is beyond doubt that more young specimens are needed to sort out the presumed synonymy of *P. annulatus* and *P. croceus*.

Based on SEM, Pegler & Young (1981) have only reported smooth spored taxa in *Pulveroboletus*. This is also the case for most African types of the genus kept at BR (unpublished data). Nevertheless *P. annulatus* and *P. croceus* were

erroneously described as smooth-spored taxa (Heinemann 1951) because SEM was not available at that time. Our EM-scanning pictures of their type specimens (respectively *Goossens-Fontana* 976, BR and *Goossens-Fontana* 740, BR) revealed that both have a rough surface, very similar to the ones from *P. bembae* and *P. luteocarneus*. Adopting these four rough-spored taxa in *Pulveroboletus* can be considered as an extension to the circumscription of the genus. Further research is needed to decide whether these four, relatively slender and fragile species, belong in a well-defined group, i.e. a separate section of *Pulveroboletus*, characterized by rough spores.

Under the scanning electron microscope the spore surface of *P. bembae* and *P. luteocarneus* (Fig. 7) is comparable to that of *Austroboletus niveus* from New Zealand (Pegler & Young 1981: 135, fig. 139). The affiliation of both new taxa to *Austroboletus* is however unlikely because of the sharp difference in spore colour, i.e. vinaceous, purplish brown or pink in *Austroboletus.*

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Literature cited

Corner EJH. 1972. *Boletus* in Malaysia. Singapore, Botanic Gardens.

Heinemann P. 1951. Champignons récoltés au Congo Belge par Madame Goossens-Fontana 1. *Boletineae*. Bull. Jard. Bot. Etat, Brux. 21(3–4): 223–346.

Heinemann P. 1964. *Boletineae* du Katanga. Bull. Jard. Bot. Etat, Brux. 34(4): 425–478.

Holmgren PK, Holmgren NH, Barnett LC. 1990. Index herbariorum. Part I: Herbaria of the World. 8th ed. Bronx, New York Botanical Garden.

Kornerup A, Wanscher JH. 1983. Methuen Handbook of Colour. 3rd ed. London, Eyre Methuen.

Murrill W.A. 1909. The *Boletaceae* of North America – I. Mycologia 1(1): 4–18.

Pegler DN, Young TWK. 1981. A natural arrangement of the *Boletales*, with reference to spore morphology. Trans. Br. Mycol. Soc. 76(1): 103–146.

Singer R. 1986. The *Agaricales* in modern taxonomy. 4th ed. Koenigstein, Koeltz Scientific Books.

Watling R, Turnbull E. 1992. Boletes from South and East Central Africa. 1. Edinb. J. Bot. 49(3): 343–361.

Watling R. 2008. A manual and source book on the boletes and their allies. Synopsis Fungorum 24. Fungiflora.